

AMENDMENTS TO THE CLAIMS

The following claims will replace all prior versions and listings of claims in this application.

1. (Currently amended) A method for producing a recombinant glycoprotein ~~comprising a desired N-glycan~~ in a ~~lower~~ non-human eukaryotic host cell which produces N-glycans comprising $\text{Man}_5\text{GlcNAc}_2$ or $\text{GlcNAcMan}_5\text{GlcNAc}_2$ structures, the method comprising the step of expressing in the host cell a nucleic acid encoding a mannosidase enzyme that is capable of hydrolyzing *in vivo* an oligosaccharide substrate comprising either or both a $\text{Man}\alpha 1,3$ and $\text{Man}\alpha 1,6$ glycosidic linkage to the extent that at least 10% of the $\text{Man}\alpha 1,3$ and/or $\text{Man}\alpha 1,6$ linkages of the substrate are hydrolyzed *in vivo*, whereby expression of said mannosidase produces one or more desired N-glycan structures on a recombinant glycoprotein expressed in said host cell, the one or more desired N-glycan structures selected from the group consisting of $\text{Man}_3\text{GlcNAc}_2$, $\text{GlcNAcMan}_3\text{GlcNAc}_2$ and $\text{Man}_4\text{GlcNAc}_2$.

2. (Currently amended) A method for producing a recombinant glycoprotein ~~comprising a desired N-glycan~~ in a ~~lower~~ non-human eukaryotic host cell which produces N-glycans comprising $\text{Man}_5\text{GlcNAc}_2$ or $\text{GlcNAcMan}_5\text{GlcNAc}_2$ structures, the method comprising the step of expressing in the host cell a nucleic acid encoding a mannosidase enzyme that is capable of hydrolyzing *in vivo* an oligosaccharide substrate comprising either or both a $\text{Man}\alpha 1,3$ and $\text{Man}\alpha 1,6$ glycosidic linkage, whereby expression of said mannosidase produces one or more desired N-glycan structures on a recombinant glycoprotein expressed in said host cell, the one or more desired N-glycan structures selected from the group consisting of $\text{Man}_3\text{GlcNAc}_2$, $\text{GlcNAcMan}_3\text{GlcNAc}_2$ and $\text{Man}_4\text{GlcNAc}_2$ and wherein the desired N-glycan is produced within the host cell at a yield of at least 10 mole percent.

3. (Currently canceled) ~~The method of claim 1 or 2, wherein the desired N-glycan produced is selected from the group consisting of $\text{Man}_3\text{GlcNAc}_2$, $\text{GlcNAcMan}_3\text{GlcNAc}_2$ and $\text{Man}_4\text{GlcNAc}_2$.~~

4. (Previously amended) The method of claim 1 or 2, wherein the desired N-glycan is characterized as having at least the oligosaccharide branch $\text{Man}\alpha 1,3 (\text{Man}\alpha 1,6) \text{Man}\beta 1,4\text{-GlcNAc}\beta 1,4\text{-GlcNAc-Asn}$.

5. (Previously amended) The method of claim 1 or 2, wherein the mannosidase enzyme is capable of hydrolyzing *in vivo* both $\text{Man}\alpha 1,3$ and $\text{Man}\alpha 1,6$ linkages of an oligosaccharide substrate comprising a $\text{Man}\alpha 1,3$ and $\text{Man}\alpha 1,6$ glycosidic linkage.

6. (Original) The method of claim 1 or 2, wherein the oligosaccharide substrate is characterized as $\text{Man}\alpha 1,3 (\text{Man}\alpha 1,6 \text{Man}\alpha 1,6) \text{Man}\beta 1,4\text{-GlcNAc}\beta 1,4\text{-GlcNAc-Asn}$; $\text{Man}\alpha 1,3 (\text{Man}\alpha 1,3 \text{Man}\alpha 1,6) \text{Man}\beta 1,4\text{-GlcNAc}\beta 1,4\text{-GlcNAc-Asn}$; $\text{GlcNAc}\beta 1,2 \text{Man}\alpha 1,3 (\text{Man}\alpha 1,6 \text{Man}\alpha 1,6) \text{Man}\beta 1,4\text{-GlcNAc}\beta 1,4\text{-GlcNAc-Asn}$; $\text{GlcNAc}\beta 1,2 \text{Man}\alpha 1,3 (\text{Man}\alpha 1,3 \text{Man}\alpha 1,6) \text{Man}\beta 1,4\text{-GlcNAc}\beta 1,4\text{-GlcNAc-Asn}$; $\text{Man}\alpha 1,3 (\text{Man}\alpha 1,3 \text{Man}\alpha 1,6 \text{Man}\alpha 1,6) \text{Man}\beta 1,4\text{-GlcNAc}\beta 1,4\text{-GlcNAc-Asn}$; $\text{GlcNAc}\beta 1,2 \text{Man}\alpha 1,3 (\text{Man}\alpha 1,3 \text{Man}\alpha 1,6 \text{Man}\alpha 1,6) \text{Man}\beta 1,4\text{-GlcNAc}\beta 1,4\text{-GlcNAc-Asn}$; $\text{Man}\alpha 1,2 \text{Man}\alpha 1,3 (\text{Man}\alpha 1,3 \text{Man}\alpha 1,6 \text{Man}\alpha 1,6) \text{Man}\beta 1,4\text{-GlcNAc}\beta 1,4\text{-GlcNAc-Asn}$; $\text{Man}\alpha 1,2 \text{Man}\alpha 1,3 (\text{Man}\alpha 1,3 \text{Man}\alpha 1,6) \text{Man}\beta 1,4\text{-GlcNAc}\beta 1,4\text{-GlcNAc-Asn}$; $\text{Man}\alpha 1,2 \text{Man}\alpha 1,3 (\text{Man}\alpha 1,3 \text{Man}\alpha 1,6 \text{Man}\alpha 1,6) \text{Man}\beta 1,4\text{-GlcNAc}\beta 1,4\text{-GlcNAc-Asn}$ or high mannan.

7. (Previously amended) The method of claim 1 or 2, wherein the mannosidase enzyme is a Class 2 mannosidase enzyme.

8. (Previously amended) The method of claim 7, wherein the Class 2 mannosidase enzyme has a substrate specificity for $\text{GlcNAc}\beta 1,2 \text{Man}\alpha 1,3 (\text{Man}\alpha 1,6 \text{Man}\alpha 1,6) \text{Man}\beta 1,4\text{-GlcNAc}\beta 1,4\text{-GlcNAc-Asn}$; $\text{GlcNAc}\beta 1,2 \text{Man}\alpha 1,3 (\text{Man}\alpha 1,3 \text{Man}\alpha 1,6) \text{Man}\beta 1,4\text{-GlcNAc}\beta 1,4\text{-GlcNAc-Asn}$; or $\text{GlcNAc}\beta 1,2 \text{Man}\alpha 1,3 (\text{Man}\alpha 1,3 \text{Man}\alpha 1,6 \text{Man}\alpha 1,6) \text{Man}\beta 1,4\text{-GlcNAc}\beta 1,4\text{-GlcNAc-Asn}$.

9. (Previously amended) The method of claim 7, wherein the Class 2 mannosidase enzyme is one which is normally found in the Golgi apparatus of a higher eukaryotic host cell.

10. (Currently amended) The method of claim 1 or 2, wherein the mannosidase enzyme is comprises a Class IIx mannosidase activity.

11. (Previously amended) The method of claim 10, wherein the Class IIx mannosidase enzyme has a substrate specificity for $\text{Man}\alpha 1,3$ ($\text{Man}\alpha 1,6$ $\text{Man}\alpha 1,6$) $\text{Man}\beta 1,4$ -GlcNAc $\beta 1,4$ -GlcNAc-Asn; $\text{Man}\alpha 1,3$ ($\text{Man}\alpha 1,3$ $\text{Man}\alpha 1,6$) $\text{Man}\beta 1,4$ -GlcNAc $\beta 1,4$ -GlcNAc-Asn; or $\text{Man}\alpha 1,2$ $\text{Man}\alpha 1,3$ ($\text{Man}\alpha 1,3$ $\text{Man}\alpha 1,6$ $\text{Man}\alpha 1,6$) $\text{Man}\beta 1,4$ -GlcNAc $\beta 1,4$ -GlcNAc-Asn.

12. (Currently amended) The method of claim 1 or 2, wherein the mannosidase enzyme is comprises a Class III mannosidase activity.

13. (Previously amended) The method of claim 12, wherein the Class III mannosidase enzyme has a substrate specificity for ($\text{Man}\alpha 1,6$ $\text{Man}\alpha 1,6$) $\text{Man}\beta 1,4$ -GlcNAc $\beta 1,4$ -GlcNAc-Asn; ($\text{Man}\alpha 1,3$ $\text{Man}\alpha 1,6$) $\text{Man}\beta 1,4$ -GlcNAc $\beta 1,4$ -GlcNAc-Asn; or high mannans.

14. (Previously amended) The method of claim 1 or 2, wherein the mannosidase enzyme is overexpressed.

15. (Previously amended) The method of claim 1 or 2, wherein the mannosidase enzyme is further capable of hydrolyzing a $\text{Man}\alpha 1,2$ linkage.

16. (Previously amended) The method of claim 1 or 2, wherein the mannosidase enzyme has a pH optimum of from about 5.0 to about 8.0.

17. (Currently canceled) ~~The method of claim 1 or 2, wherein the mannosidase enzyme is further capable of hydrolyzing a $\text{Man}\alpha 1,2$ linkage.~~

18. (Previously amended) The method of claim 1 or 2, wherein the mannosidase enzyme is localized within the secretory pathway of the host cell.

19. (Previously amended) The method of claim 1 or 2, wherein the mannosidase enzyme is localized within at least one of the ER, Golgi apparatus or the trans Golgi network of the host cell.

20. (Previously amended) The method of claim 1 or 2, wherein the nucleic acid encoding the mannosidase enzyme encodes an enzyme comprising a mannosidase catalytic domain fused to a targeting peptide.

21. (Previously amended) The method of claim 20, wherein the mannosidase catalytic domain is native to the host cell.

22. (Previously amended) The method of claim 20, wherein the mannosidase catalytic domain is heterologous to the host cell.

23. (Previously amended) The method of claim 1 or 2, wherein the mannosidase enzyme is selected from the group consisting of *Arabidopsis thaliana* Mannosidase II, *C. elegans* Mannosidase II, *Ciona intestinalis* mannosidase II, *Drosophila* mannosidase II, Human mannosidase II, Mouse mannosidase II, Rat mannosidase II, Human mannosidase IIx, Insect cell mannosidase III, Human lysosomal mannosidase II and Human cytoplasmic mannosidase II.

24. (Previously amended) The method of claim 20, wherein the targeting peptide is native to the host cell.

25. (Previously amended) The method of claim 20, wherein the targeting peptide is heterologous to the mannosidase catalytic domain.

26. (Original) The method of claim 1 or 2, further comprising the step of isolating the glycoprotein from the host cell.

27. (Original) The method of claim 1 or 2, wherein the host cell is selected from the group consisting of *Pichia pastoris*, *Pichia finlandica*, *Pichia trehalophila*, *Pichia koclamae*, *Pichia membranaefaciens*, *Pichia opuntiae*, *Pichia thermotolerans*, *Pichia salictaria*, *Pichia guercuum*, *Pichia pijperi*, *Pichia stiptis*, *Pichia methanolica*, *Pichia sp.*, *Saccharomyces cerevisiae*, *Saccharomyces sp.*, *Hansenula polymorpha*, *Kluyveromyces sp.*, *Kluyveromyces lactis*, *Candida albicans*, *Aspergillus nidulans*, *Aspergillus niger*, *Aspergillus oryzae*, *Trichoderma reesei*,

Chrysosporium lucknowense, *Fusarium sp.*, *Fusarium gramineum*, *Fusarium venenatum* and *Neurospora crassa*.

28. (Original) The method of claim 27, wherein the host cell is *Pichia pastoris*.

29. (Original) The method of claim 1 or 2, wherein the glycoprotein is a therapeutic protein.

30. (Original) The method of claim 29, wherein the therapeutic protein is selected from the group consisting of erythropoietin, cytokines, coagulation factors, soluble IgE receptor α -chain, IgG, IgG fragments, IgM, interleukins, urokinase, chymase, urea trypsin inhibitor, IGF-binding protein, epidermal growth factor, growth hormone-releasing factor, annexin V fusion protein, angiostatin, vascular endothelial growth factor-2, myeloid progenitor inhibitory factor-1, osteoprotegerin, α -1-antitrypsin and α - feto protein.

31 – 56. (Withdrawn)